Mumps ELISA
IgG / IgM Testkit

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FOR IN VITRO DIAGNOSIS ONLY

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1. Intended Use

The Mumps ELISA IgG / IgM is intended for the semiquantitative and qualitative detection of IgG- and IgM-antibodies against Mumps viruses in human serum. The IgM-antibody determination is used for diagnosing acute infections and the IgG-antibody determination for detecting the serostatus.

2. Diagnostic Relevance

The mumps virus belongs to the family of the Paramyxoviridae. The Paramyxoviridae also include measles, parainfluenza and RSV. Humans are the only natural host for the mumps virus, which is distributed worldwide. (1)

In the developed countries of the northern hemisphere, the highest incidence is among 5- to 9-year old children. Above the age of 15 years, 80-90% have been infected. As the contagiousness of mumps viruses is only 40% compared to measles and varicellas, disease occurs in adults about three times more often than with measles. (1) About 30-50% of infections have a silent course and life-long immunity is usually acquired following infection. (2,4)

The mumps virus is spread from person to person through saliva droplets or nasal secretion. (1) The incubation period is 18-21 days, after which the classical lead symptom of unilateral or bilateral febrile parotitis occurs with obvious swelling. (3)

Most mumps virus infections (synonym: epidemic parotitis) follow a course of generalised self-limiting illness. However, complications can occur, particularly with increasing age. Orchitis, which is usually unilateral, occurs in about 20% of postpubertal patients. If it is bilateral, there is a danger of testicular atrophy with subsequent infertility. In 10% of cases in the first decade of life, meningitis can occur as a complication, usually benign but with the rare late sequela of deafness. In the first trimester of pregnancy, there is a risk of abortion; however, embryopathy is unknown. Other possible complications such as epidydimitis, oophoritis, meningoencephalitis, mastitis or pancreatitis should be noted. (1)

The disease always commences with fever and in most cases with unilateral parotid swelling. This is easy to recognise by lifting the earlobe. In 2/3 of cases, the other side is also affected later. Occasionally, only the submaxillary and sublingual glands are affected without discernible parotid involvement. (1)

Referring to Buxbaum still more than 20% of the 15 to 19 year old teenager show a lack of immunity against the mumps virus. Serum samples with a rate of >90% (5) of protective antibodies are only found in the population aged 40 years or older.

Vaccination against mumps is generally recommended, and is usually carried out with a live vaccine (MMR). With this vaccine the conversion rate is 95%. Vaccination should be considered for children and exposed adults. (1)

The diagnosis is made from the lead symptom of parotitis. The virus can be isolated from saliva samples. (1) Serological test systems, in particular, are suitable for laboratory diagnosis. ELISA, KBR or HHT are employed. The virus can also be detected directly by PCR. Parotitis from other causes such as Coxsackie viruses, EBV, parainfluenza, influenza A or adenoviruses must be considered in the differential diagnosis. Bacterial pathogens such as staphylococci or atypical mycobacteria and also fungal infections should also be included in the differential diagnosis. (1,4)

ELISA test systems are particularly suitable for routine use in serology to distinguish the different immunoglobulin classes. Acute mumps virus infection can be confirmed even in the first days of illness by the detection of specific IgM antibodies. (3) The specific IgM antibodies are usually detectable for a month (3). IgG antibodies are also detectable two weeks after the start of the illness (3) and persist for many years (4).

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents (IgG and IgM Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use

5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgM negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates

7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the Sekisui Virotech user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The Sekisui Virotech System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 Virotech ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- and IgM-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All Sekisui Virotech ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

Sekisui Virotech recommends the following procedure:

1. Sekisui Virotech recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgM-antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) Virotech Units (VE)

The Virotech Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the Virotech Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$\text{VE (positive control)} = \frac{\text{OD (positive control)}}{\text{OD (cut-off control)}} \times 10$$

$$\text{VE (patient serum)} = \frac{\text{OD (patient serum)}}{\text{OD (cut-off control)}} \times 10$$

9.3 Interpretation Scheme IgG and IgM

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. Anti-doublestrand DNA (α–dsDNA) sera (ANA, systemic lupus erithematodes) show crossreactivity to the Sekisui Virotech MUMPS ELISA.

10. Performance Data

10.1 Sensitivity and Specificity

To determine the analytical sensitivity and specificity of Mumps IgG 302 sera were tested in an internal study. Two ELISAs of different suppliers were used as reference test. The serapanel contains sera routinely used by the virology of the University Cologne.

Reference ELISA	VT Mumps IgG Elisa		
	negative	borderline	positive
negative	72	0	0
borderline	2	2	0
positive	12	8	206

12 sera could neither in the Virotech ELISA nor in the two reference tests be clearly defined. They are not included in the calculation of sensitivity and specificity.

The Virotech Mumps IgG ELISA shows following values:
Sensitivity 94,5%
Specificity >99,8%

To determine the analytical sensitivity and specificity of Mumps IgM 258 sera were tested in an internal study. Two ELISAs of different suppliers were used as reference test. The serapanel contains sera routinely used by the virology of the University Cologne.

Reference ELISA IgM n= 258	VT Mumps IgM Elisa		
	negative	borderline	positive
negative	237	0	1
borderline	5	0	0
positive	1	1	13

6 sera could neither in the Virotech ELISA nor in the two reference tests be clearly defined. They are not included in the calculation of sensitivity and specificity.

The Virotech Mumps IgM ELISA shows following values:
Sensitivity 92,9%
Specificity 99,6%

10.2 Prevalence (Expected Values)

To the determine the Expected Values 80 blood bank sera were tested in IgG and IgM.

(n=80)	**IgG**		**IgM**	
	number	**(%)**	**number**	**(%)**
negative	20	25,0	79	98,7
borderline	7	8,7	1	1,3
positive	53	66,3	0	0,0

A further IgG testing resulted in in comparison to a ELISA reference test the following result:

(n=40)	IgG	
	(%) Virotech	(%) ELISA reference test
negative	30,0	20,0
borderline	5,0	25,0
positive	65,0	55,0

10.3 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested with the same serum sample. The obtained coefficient of variation is lower then 9%.

10.4 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 10 independent test runs by different persons in different laboratories.
The obtained variation coefficient values are lower than 15%.

11. Literature

1. H. Hahn et al.; Medizinische Mikrobiologie; 3. Auflage 2001, S. 554-556
2. T. Porstmann; Diagnostische Bibliothek Nr. 19, 1994
3. F. Burkhardt et al.; Mikrobiologische Diagnostik; 1992, S. 362-363
4. H. Reploh et al.; Lehrburch der Medizinischen Mikrobiologie; 7. Auflage 1994, S. 838
5. http://www.aerzte-zeitung.de/docs/2002/01/22/012a1301.asp

Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ **IgG-Samples – Dilution 1:101**

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

▼ **IgM-Samples - Dilution 1:101**
Rheumafactor-absorption with RF-SorboTech

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇩ Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG, IgM
⇩ Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇩ Stopping		**50 µl Stopping Solution** shake carefully
⇩ Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**